GP 1645

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Ara HOVANESSIAN et al.	Group Art Unit: 1645
Application No.: 09/825,886	Prior Appln.
	Examiner: Zeman, R.
Filed: April 5, 2001	
For: A NOVEL CELL SURFACE RECEPTOR FOR HIV RETROVIRUSES, THERAPEUTIC AND DIAGNOSTIC USES	

Box PG-PUB
Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

REQUEST FOR CORRECTED PATENT APPLICATION PUBLICATION UNDER 37 C.F.R. § 1.221(b)

On June 20, 2002, the U.S. Patent and Trademark Office published this application as Publication No. US 2002/0076693 A1. The published application contains a mistake that is the fault of the Office and is, in Applicants' view, material. Attached hereto is a copy of the relevant page of the originally filed application and a marked-up copy of the corresponding page of the published application containing the mistake.

A mistake is material when it affects the public's ability to appreciate the technical disclosure of the patent application publication or determine the scope of the provisional rights that Applicants may seek to enforce upon issuance of a

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patent. *See* 37 C.F.R. § 1.221(b). The mistake listed below may affect the public's ability to appreciate the technical disclosure of the patent application publication or to determine the scope of provisional rights.

The mistake, which is indicated in red ink on the relevant page of the marked-up copy of the published application attached hereto, is listed below with its corrections.

1. After paragraph 0477, the publication provides an incomplete version of Figure 49. Three lines of text are missing from Figure 49 in the published application, as provided below:

III. Aminoacid and cDNA sequence of P30/PHAPI

IV. Aminoacid and cDNA sequence of P40/PHAPII

V. cDNA sequence of nucelolin/P95

This text is included in the originally filed application on the bottom of page 87. The Office appears to have omitted the recited text from Figure 49. This mistake is clearly material as the omission of this text necessarily impedes the public's ability to appreciate the technical disclosure of the patent application publication. For at least this reason, this mistake should be corrected.

Applicants request that the Office correct the above-identified mistake in the published application, which were the fault of the Office. Further, Applicants request that the Office forward to Applicants a copy of the corrected published

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application or at least a notification of the occurrence or predicted occurrence of the corrected publication once it has been corrected.

Applicants believe that no Petition or fee is due in connection with this Request; however, if any Petition or fee is due, please grant the Petition and charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: August 20, 2002

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Enclosures:
Copy of the relevant page of the originally filed application; marked-up copy of corresponding page of the published application

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proteins, wild type (lanes 1,3, 5) and deleted (lanes 2, 4, 6), were analyzed by SDS/PAGE. Such samples were analyzed by immunoblotting using the rabbit polyclonal antibodies raised against the synthetic N-terminal peptide corresponding to the PHAP I sequence (1/500^e dilution of serum; lanes 1,2), or by ligand-blotting (Callebaut et al., 1997) in the presence of either the biotin-labeled 5[Kψ (CH_2N)PR]-TASP (5μM; lanes 3, 4) or the biotin-labeled V3 loop peptide (25 μM; lanes 5, 6). The antibodies and the biotin-labeled molecules were revealed with specific immunoglobulins labeled with horseradish peroxidase (-HLP) and streptavidin-HRP respectively (amersham). The numbers on the left give the position of molecular weight (in kDa) protein markers. Material corresponding to 1μg protein was analyzed in each lane.

We have previously suggested that the capacity of nucleolin, PHAP II, and PHAP I to bind 5[Kψ (CH_2N)PR]-TASP and gp120 is due to the presence of acidic domains (amino acids glutamate and aspartate) in these V3 loop binding proteins. Here we demonstrate that recombinant PHAP I binds 5[Kψ (CH_2N)PR]-TASP or the V3 loop, however PHAP I devoid of its C-terminal acidic domain does not bind. These results therefore illustrate that the acidic domain could indeed account for the capacity of the V3 loop binding proteins to bind 5[Kψ (CH_2N)PR]-TASP or the V3 loop.

Figure 49 :

I. Aminoacid and II genomic DNA sequences and mRNA of the P95/nucleolin protein.

exons : 1070. .1198, 2159. .2275, 3439. .3916,4587. .4784,4889. .4975,5160. .5301,6307. .6431,7037. .7160,7620. .7777,8292. .8415,8652. .8785,9279. .9405,9792. .10006,10140.10499

mRNA join(1070. .1198,2159. .2275,3439. .3916,4587. .4784,4889. .4975,5160. .5301,6307. .6431,7037. .7160,7620. .7777,8292. .8415,8652. .8785,9279. .9405,9792. .10006,10140. .10499)

CDS join(1181. .1198,2159. .2275,3439. .3916,4587. .4784,4889. .4975,5160. .5301,6307. .6431,7037. .7160,7620. .7777,8292. .8415,8652. .8785,9279. .9405,9792. .10006,10140. .10216)

III Aminoacid and cDNA sequence of P30/PHAPI

IV. Aminoacid and cDNA sequence of P40/PHAPII

V. cDNA sequence of nucleolin/P95

an association of these molecules. The experimental conditions are identical to those described in the legend of FIG. 46 .

[0475] FIG. 48 : 5[Kψ (CH_2N)PR]-TASP and the V3 loop peptide do not bind PHAP-I deleted in its acidic region.

[0476] Wild type (aa 1-249) and deleted (aa 1-167) PHAP I both fused with the Histidine Tag His_6 were produced in the yeast system *Pichia pastoris* expression system (Invitrogen), and purified with Ni^{2+} charged columns according to manufacturor's instructions (Ni—NTA, QIAGEN). Aliquots of the purified proteins, wild type (lanes 1, 3, 5) and deleted (lanes 2, 4, 6), were analyzed by SDS/PAGE. Such samples were analyzed by immunoblotting using the rabbit polyclonal antibodies raised against the synthetic N-terminal peptide corresponding to the PHAP I sequence (1/500^e dilution of serum; lanes 1, 2), or by ligand-blotting (Callebaut et al., 1997) in the presence of either the biotin-labeled 5[Kψ (CH_2N)PR]-TASP (5 μM; lanes 3, 4) or the biotin-labeled V3 loop peptide (25 μM; lanes 5, 6). The antibodies and the biotin-labeled molecules were revealed with specific immunoglobulins labeled with horseradish peroxidase (-HLP) and streptavidin-HRP respectively (amersham). The numbers on the left give the position of molecular weight (in kDa) protein markers. Material corresponding to 1 μg protein was analyzed in each lane.

[0477] We have previously suggested that the capacity of nucleolin, PHAP II, and PHAP I to bind 5[Kψ (CH_2N)PR]-TASP and gp120 is due to the presence of acidic domains (amino acids glutamate and aspartate) in these V3 loop binding proteins. Here we demonstrate that recombinant PHAP I binds 5[Kψ (CH_2N)PR]-TASP or the V3 loop, however PHAP I devoid of its C-terminal acidic domain does not bind. These results therefore illustrate that the acidic domain could indeed account for the capacity of the V3 loop binding proteins to bind 5[Kψ (CH_2N)PR]-TASP or the V3 loop.

Figure 49

I. Aminoacid and II genomic DNA sequences and mRNA of the P95/nucleolin protein.

exons	: 1070. .1198, 2159. .2275, 3439. .3916,4587. .4784,4889. .4975,5160. .5301,6307. .6431,7037. .7160,7620. .7777,8292. .8415,4852. .8785,9279. .9405,9792. .10006,10140.10499
mRNA	join(1070. .1198,2159. .2275,3439. .3916,4587. .4784,4889. .4975,5160. .5301,6307. .6431,7037. .7160,7620. .7777,8292. .8415,8652. .8785,9279. .9405,9792. .10006,10140. .10499)
CDS	join(1181. .1198,2159. .2275,3439. .3916,4587. .4784,4889. .4975,5160. .5301,6307. .6431,7037. .7160,7620. .7777,8292. .8415,8652. .8785,9279. .9405,9792. .10006,10140. .10216)

III. Aminoacid and cDNA sequence of P30/PHAPI

IV. Aminoacid and cDNA sequence of P40/PHAP II

V. cDNA sequence of nucleolin/P95

[0478] Materials and Methods

[0479] I. Materials

[0480] Recombinant gp120 and gp41 corresponded to the external and transmembrane envelope glycoprotein, respectively, of HIV-1 Lai (IIIB), purchased from Neosystem Laboratories, Strasbourg. Recombinant gp120 is produced by the baculovirus expression system, whereas recombinant gp41 was produced by the *E. coli* expression system. Recombinant soluble CD4 was produced in baculovirus expression sysem and was purchased from Neosystem. Other recombinant preparations of gp120 corresponding to that of HIV-1 isolates, MN, SF2 (from Dr. K. Steimer, Chiron Corporation), LA V(or Lai), and the nonglycosylated gp120 of HIV-1 SF2 (Env 2-3; from Dr. K. Steimer; Chiron Corporation) were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. The gp120 MN and LA V are produced in insect cells using the baculovirus expression system, gp120 SF2 is produced in CHO cells, whereas the nonglycosylated gp120 SF2 is produced in the yeast.

[0481] A. Antibodies

[0482] The monoclonal antibody (mAb) CC98 against human nucleolin (Chen et al., 1991; Fang and Yeh, 1993) was generously provided by Dr. N.-H. Yeh, Graduate School of Microbiology and Immunology, National Yang-Ming Medical College, Shih-Pai, Taiwan, Republic of China. Rabbit antiserum raised against a purified preparaion of human nucleolin was generously provided by Drs. M. Erard and C. Faucher, Centre de Recherche de Biochimie et de Génétique Cellulaire du CNRS, Toulouse, France. The mAb specific to human CD4 and reacting with the gp120 binding domain was kindly provided by Dr. E. Bosmans (Eurogenetics, Tessenderlo, Belgium). Another mAb specific to human CD4 and reacting with the gp120 binding domain, mAb OKT4A, was purchased from Ortho Diagnostics Systems. The mAb specific for histone H3 was produced in the laboratory (Benkirane et al., 1996). MAb N11/20 against the V3 loop of gp120, mAb 110/C against an epitope in gp120 corresponding to fragment 282-284 amino acids, mAb 110/D against an epitope situated at residues 381-394, mAb 41-A against gp41 (both gp120 and gp41 of HIV-1), and mAb 125-A against the extenal envelope glycoprotein of HIV-2 were provided by Dr. J. C. Mazie, Hybridolab, Institut Pasteur. MAb 110-4 against the V3 loop and mAb 110-1 against the C-terminal domain of gp120 (Kinney-Thomas et al., 1988; Linsley et al., 1988) were obtained from Genetics Systems (Seattle, Wash.). MAb ADP390 against the CD4 binding domain in gp120 (from Drs. J. Cordell and C. Dean) was provided by MRC AIDS Directed Programme Reagent Repository (McKeating et al., 1992). MAb AD3 against the first 204 amino acids of gp120 (From Drs. K. Ugen and D. Weiner), mAb V3-21 against the INCTRPN sequence at residues 298-304 containing the N-terminal end of the V3 loop (from Dr. J. Laman), and MAb b12 against the CD4 binding domain in gp120 (from Drs. D. Burton and C.